RELATING DNA AND RNA BIOMARKERS TO MICROCYSTIN LEVELS IN CAYUGA LAKE HARMFUL ALGAL BLOOMS AND LAB STUDIES

A Thesis

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Master of Science

by

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ABSTRACT

Cyanobacteria harmful algal blooms (cHABs) are expanding globally in freshwaters including the Finger Lakes region of New York State. cHABs can express toxins that pose a threat to public health via recreational or drinking water exposure. Current toxic HAB monitoring in New York State requires microcystin (MC) quantification by certified "ELAP" labs performing mass spectrometry or ELISA based methods. However, tests cost $150-$200 per sample and the limited number of certified facilities statewide lead to long lag times in getting results of testing (>1 day). There is value in testing new screening tools that correlate with microcystin levels but are cheaper and more rapid and can be performed in the field by common citizens without formal scientific training, such as those who volunteer to perform HAB monitoring in their home watersheds, such as the new HAB Harriers of Cayuga Lake.

Here in this study, prokaryotic 16S ribosomal RNA gene (16S rRNA) Illumina sequencing was used to determine the community profiles in two Finger Lakes, Cayuga Lake and Honeoye Lake during late summer 2018. The microcystin synthesis pathway genes (mcy genes) of *Microcystis*, the most common genus associated with toxic HABs, are well studied and can be indicative of the presence of potentially toxic cyanobacterial strains and mcy gene transcripts can indicate expression of the MC synthesis pathway. Therefore, we targeted the Microcystis mcyA gene to quantify the corresponding gene and transcripts levels using qPCR and RT-qPCR. Correlation between potential biomarkers, mcyA DNA and RNA levels, with microcystins (MCs) were investigated in both field samples and lab cultures with a model pure culture, *Microcystis aeruginosa* PCC7806 to determine the best indicators for MC levels. A portable qPCR device, the Biomeme two3 was investigated for a rapid (<1 hour) on-site test for field samples. Additionally,

monoculture experiments of *Microcystis aeruginosa* PCC 7806 were conducted under various N:P ratios to investigate impact of nutrients on mcy gene copies and transcripts as well as MC synthesis. The sequencing results indicated the two lakes had distinct community structures. Cyanobacteria were the most abundant phylum (59%) among the bacterial communities of Cayuga Lake, with *Microcystis* being the dominant MC producing genus (82% of cyanobacteria). Honeoye Lake had more *Synechocystis* (48% of cyanobacteria) than *Microcystis*. Although both mcyA DNA and RNA levels had a significant correlation with MCs in lab experiments, in Cayuga 2018 bloom samples (August/September) and 2019 pre-bloom samples (April), only mcyA RNA levels showed significant positive correlations with MC concentrations, while mcyA DNA levels did not, suggesting mcyA transcript levels could be better indicators of potential risks from MCs than mcyA gene levels. The handheld portable Biomeme two3 qPCR device would be promising in further lake monitoring due to its rapid procedure (< 1 hour), reasonable cost ($~40 per test) and inclusion of an Internal Positive Control (IPC) for qPCR inhibition testing to flag false negatives or underestimated gene or transcripts levels.

Key words: *Microcystis*, microcystin, mcyA, qPCR, RT-qPCR, community profiling, cHAB monitoring

BIOGRAPHICAL SKETCH

Lingzi Xia was born in Chongqing, China in 1995. She graduated with a bachelor's degree in Environmental Engineering from Beijing Normal University in 2017. She had a lot of research experience in molecular test of nitrogen cycle microorganisms as well as organic compounds bioaccumulation during her undergraduate studies. The two projects she worked with have been published. Those experience raised her interest in environmental science.

She entered Cornell University since 2017 August and started her study towards the M.S. Degree in Environmental Engineering. She was attracted by the free academic atmosphere in Cornell. She went to Honduras with Agua Clara team in 2018 winter and helped improving the sustainable drinking water strategies for the developing countries. She conducted experiments related to the cyanobacteria harmful algal blooms in Cayuga Lake, hoping to help monitoring cHAB events.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

mcyA	Microcystin synthetase functional gene A
cHABs	Cyanobacteria harmful algal blooms
MCs	Microcystins
MC	Microcystin
SRP	Soluble reactive phosphorus
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
CSI	Community Science Institute
CCL	Contaminant Candidate List
N	Nitrogen
P	Phosphorous
PCoA	Principal Coordinates Analysis
mcy	Microcystin synthetase
Chl-a	Chlorophyll a
cDNA	Complementary DNA obtained by reverse transcribing RNA
ELISA	Enzyme-linked immunosorbent assay
PCR	Polymerase chain reaction
qPCR	Quantitative real-time PCR
RT-qPCR	Reverse transcription quantitative PCR

CHAPTER 1: INTRODUCTION

1.1 Context

Harmful algal blooms (HABs) are receiving great concern due to its impact on ecosystem and public health. In freshwater, the most common and severe algal blooms are caused by cyanobacteria [1]. cHABs pose a threat to public health and aquatic ecosystem health, and could cause economic damages [2]. Animal deaths have been reported and the toxins produced by cyanobacteria have made a challenge to drinking water treatment facilities. cHABs have been recognized globally in waters impacted by large-scale agriculture, industrialization, and urbanization during the past few years [3].

Traditional cHAB monitoring procedure includes microscope work combined with toxin measurements which is time consuming. However, cyanobacteria bloom bursts quickly, and it is difficult to take prompt action without early warning and rapid monitoring. In order to better assess the health risks associated with the increasing cyanobacterial blooms, there is need for improved detection of toxigenic cyanobacteria.

Environmental factors that have been linked to cHAB occurrence include growth-limiting factors such as nutrients, light, temperature as well as cyanobacterial community composition, hydrologic and meteorological conditions [3]. Despite this general understanding, many unanswered questions remain about occurrence, environmental triggers for toxicity. Controversial results have been found regarding microcystin (MC) production under different nutrient conditions. More comprehensive study needs to be done to elucidate the impacts of nutrients on MC synthesis.

Current measures to reduce eutrophication and cHABs addressing the source, transport and fate of nutrients require thorough understanding of the causes and prevention of cHABs [4]. Many studies

related to the mechanism of cHAB response to environmental factors and the tools to monitor cHAB all aim to help mitigate this situation.

1.2 Research objectives

The overarching objective of this research is to have a better understanding of toxic cyanobacteria during cHAB events, through field sampling molecular test and lab simulation experiments.

This goal was attained through the following specific objectives:

I. To obtain the community profiles of the Cayuga Lake and Honeoye Lake during bloom season, such as the bacterial taxa and potential microcystin producing cyanobacteria as well as the relative abundances;

II. To investigate the correlations between mcyA DNA/RNA levels with MCs in Finger Lake 2018 summer samples and lab monoculture experiments;

III. To develop a workflow for rapid detection of mcyA genes and RNA on a handheld field-usable qPCR device

IV. To examine how mcyA gene copies and transcripts of PCC7806 change under various nutrient ratios.

1.3 Hypotheses

1.16s rRNA gene community profiling of Cayuga Lake bloom samples will inform the specific populations contributing to the bloom. Cayuga and Honeoye would have a relatively different community structure.

2. RNA transcripts of microcystin toxin production genes correlate better than DNA levels with actual microcystin levels in both lab and filed conditions.

3. Biomeme two3 would be a potable tool for qPCR test in field.

4. Excessive nitrogen results in higher microcystins level and more mcyA transcripts than in balanced N:P or P excess conditions.

CHAPTER 2: BACKGROUND

2.1 Cyanobacteria harmful algal blooms

2.1.1 Cyanobacteria

Cyanobacteria are a phylum of bacteria, also called as blue-green algae. They are photosynthetic prokaryotes that can obtain energy from light. They are highly specialized and competitive. Some of them can regulate buoyancy and some of them can fix nitrogen [5]. They form in colonies in the natural environment to protect themselves from grazing. Recently, waters impacted by large-scale agriculture and industrialization have increased the occurrence of cHABs [7]. Under favorable conditions such as excessive nutrients, cyanobacteria can not only proliferate massively to form blooms but also produce toxins that are harmful.

There are over 40 cyanobacterial genera that can produce toxins. The most widespread cyanotoxins, microcystins (MCs), are produced by several genera of cyanobacteria, including *Planktothrix*, *Microcystis*, *Anabaena*, *Nostoc, Oscillatoria,* etc. However, not all species in those genera or even not all strains in one species are able to produce MCs. The mechanisms of cyanobacteria to produce toxins are unclear so far.

Among different types of cyanobacteria, *Microcystis* is a genus that is most commonly found in New York State. There are toxic and non-toxic *Microcystis* species, but they are morphologically indistinguishable. Their appearance under microscope are usually unicellular, with sizes ranging from 1-10μm, but they usually form in colonies in the environment. Their ability to regulate buoyance among certain species due to large gas vesicles are reported to have association with their dominance in fresh water bodies, since it provides them with advantages to gain ammonium and phosphorus according to the nutrient stratification as well as allow them to take optimal light intensities and carbon dioxide levels.[5] While many have argued nitrogen fixation cyanobacteria

are more competitive when N is limited, studies have found non-nitrogen fixation *Microcystis* still dominated even under N limited condition [6].

Cyanobacteria undergo mostly asexual reproduction, binary fission. It is reported the nucleoid of most cyanobacterial cells contains a single, large, circular chromosome of double stranded DNA. *Microcystis* aeruginosa PCC7806 is a model specie in lab. Complete genomes of PCC7806 have been sequenced and annotated. Reported PCC7806 genome size was 5.17Mb. [7]

2.1.2 Cyanobacterial toxins (cyanotoxins) and genes

Cyanotoxins are sometimes thought to be secondary metabolites[8], which are not directly related to key biochemical pathways leading to growth or survival. However, some researchers thought they were not secondary metabolites since they would be involved in some essential intracellular activities.[9] The mechanisms to produce cyanotoxins are still unknown. Cyanotoxins production was thought to serve as deterrence against zooplankton grazing.[10] Among different types of toxins, MCs are the most widespread one. They are hepatotoxic cyclic peptides, which are potent and specific inhibitors of protein phosphatases, and can cause liver damage. The synthesis of MCs requires integrated steps by a group of polyketide synthase (PKS) and non-ribosomal peptidesynthetase (NRPS).[11] They are the most common toxins in New York State. There are over 50 known variants of MCs, with MC-LR being the most common one.[12]

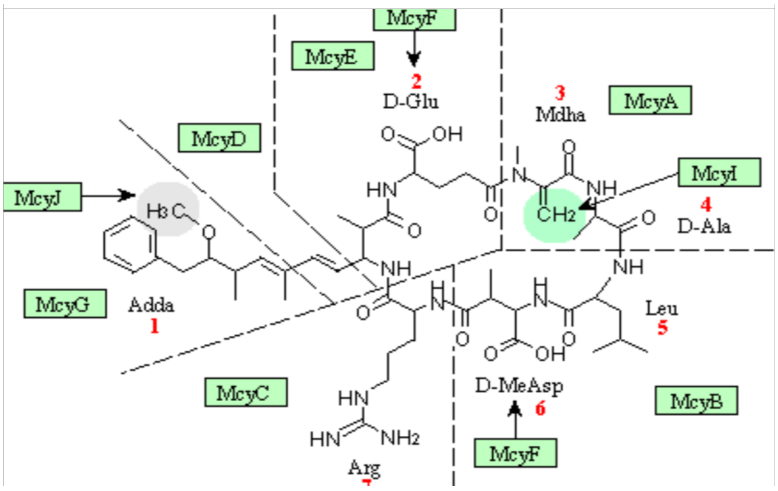

Figure 1: MC-LR pathways from KEGG. Each of the "Mcy" enzymes codes a different step in microcystin synthesis. MC-LR is an example of a non-ribosomal peptide

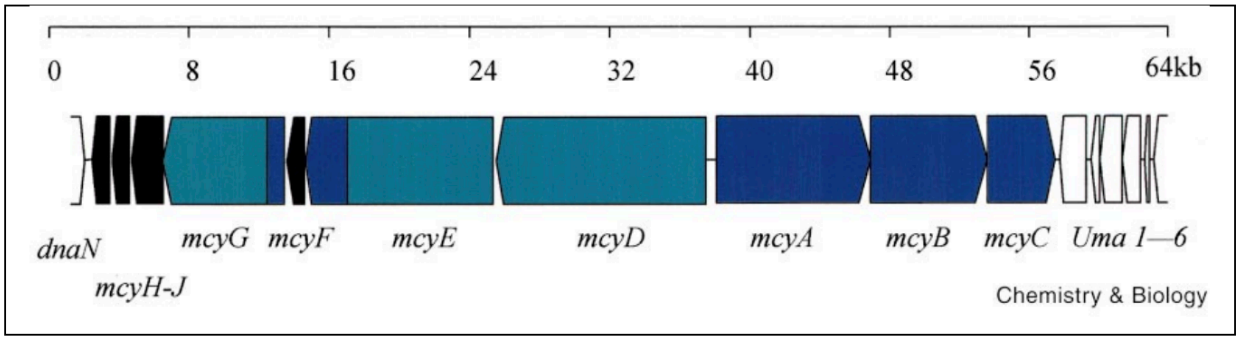

Figure 2: Organization of the gene cluster for microcystin biosynthesis. [11]

The microcystin synthetase gene cluster contains ten open reading frames (ORFs; mcyA to mcyJ) (Figure 2). Each mcy gene encodes unique Mcy proteins that are involved in synthesis or cyclization of different parts of the microcystin molecule. The mcyA gene in the mcy cluster, encodes a polyketide synthase modular involved in the addition of Adda, the amino acid which is conserved in all the microcystin variants, to the MC-LR ring structure.

Cyanotoxins pose a threat to public health via recreational or drinking water exposure. The EPA's Office of Water has listed cyanobacteria and cyanotoxins on the Contaminant Candidate List (CCL) 1 (1998), CCL 2 (2005) and microcystin-LR on the CCL 4 (2016). World Health Organization

(WHO)(2011) set 1 part per billion (ppb) of MCs for a safe drinking water limit of and 10 ppb for recreational water. The New York State Department of Environmental Conservation (NYSDEC) defines a “bloom with high toxin” as 20 ppb near the lake shore.[13] However, traditional water treatment steps have demonstrated limitations on removing MCs during heavy bloom periods, which may cause health risk for water consumers.[14]

2.2 Global and local occurrence

2.2.1 Global occurrence

Cyanobacterial blooms are a common occurrence in freshwater bodies globally. Blooms of cyanobacteria are more prevalent in eutrophic or hypereutrophic waters. In temperate lakes, blooms often occur during the late summer to early fall period whereas sub-tropical and tropical water bodies might experience this phenomenon at any time of the year [16]. Cyanobacterial blooms have been reported in almost all countries around the world. Between 10-92% (mean 59%) of blooms surveyed worldwide are toxic. Most blooms are usually made up of more than one cyanobacterial species, each often containing both toxic and non-toxic strains.[15]

Chronic cHABs have been reported throughout the United States, and cyanotoxins have been implicated in human and animal illness and death in at least 43 States. Several cHABs have gained

national attention because of their extent and severity.

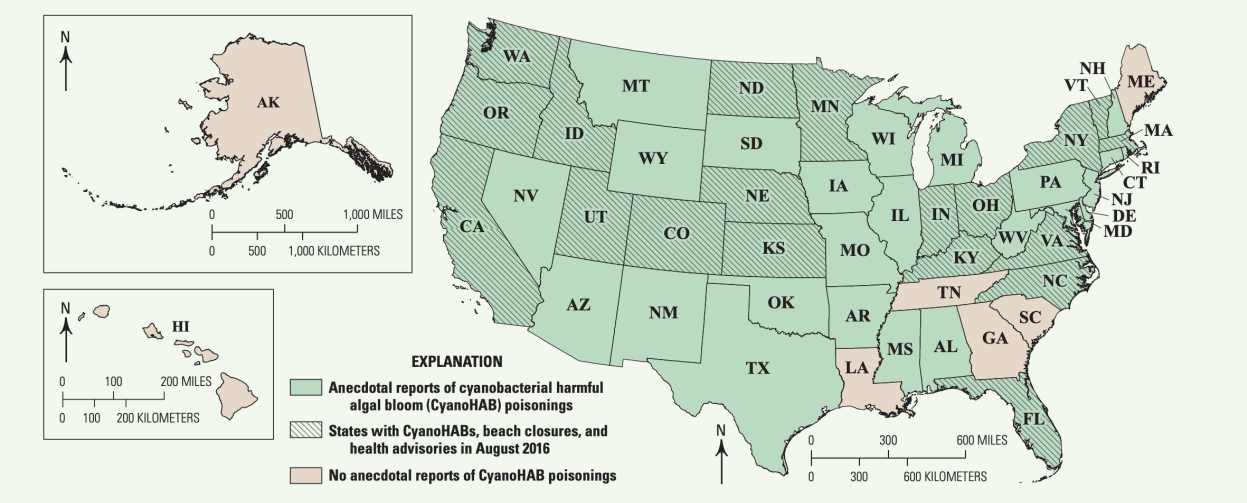

Figure 3: cHAB events in the US by USGS[16]

2.2.2 Cyanobacterial blooms in Finger Lakes (FLs)

Halfman has monitored water quality monthly from eight eastern Finger Lakes at both surface and bottom water since 2005. They conducted 9 years research to investigate the potential triggers of the onset HABs in those lakes. They have found shallower depths, higher nutrients and suspended sediment and chl-a concentrations coincided with HABs occurrence. HABs typically occurred in calm sunny conditions. More significantly, increase in P concentration in offshore surface waters suggests P is a driver of HABs. [17]

HABs are also emerging events in Finger Lakes. Since 2012, there have been reports of confirmed HABs. This is an ongoing trend, on 2017, all finger lakes have been impacted with HABs and most of them were highly toxic. In Cayuga Lake, 50 blooms were reported in 3 days in 2017.

2.3 Impacts and Causes of cHABs

2.3.1 Impacts

Blooms of cyanobacteria cause several water quality problems including but not limited to : toxin production, release of taste and odor compounds, creation of anoxic conditions during bloom die-off as well as related aesthetic issues.

Cyanobacteria produce taste-and-odor compounds. Those compounds also may also be toxic and accumulate in fish, which can negatively impact the aquaculture industry and public health. High toxins can cause death of other aquatic lives which can deteriorate aquatic ecosystems. Large blooms can deplete the waters of dissolved oxygen leading to hypoxic "dead-zones" where aquatic life cannot exist. Human consumption of swimming water that is tainted with cHABs may result in gastroenteritis, skin irritation, and allergic reactions of the skin and eyes. Long term exposure may cause liver damage. cHABs also create issues for aquatic life. Economic damages related to cHABs include loss of recreational revenue, decreased property values, and increase in drinking-water treatment costs.[18]

2.3.2 Environmental factor effects

A lot of research has been done to investigate environmental factor effects on MC synthesis. Environmental conditions such as sunlight, rising temperatures, low water turbulence, and cultural eutrophication are amongst the most common promoters of cyanobacterial growth [19], with global warming and nutrients being the most significantly attributors[20][21]. Global warming may lead to rise in toxicity level of cHAB. Rising CO_2 concentrations was reported to increase ~2.7 fold in the cyanobacterial biomass and ~2.5 fold more microcystin per cell [22]. A positive effect of PAR on microcystin production before maximum growth rate was found [23]. Hydrologic conditions such as low flushing, water column stability and meteorological conditions such as rain events, warm temperatures, and prolonged ice-free growing seasons have been proved to have an effect on cHABs occurrence.[24]

For nutrients, there have been contradictory results regarding the relationship of microcystin production with nitrogen or phosphorus. Those contradictions may be due to methodology differences or strain specific results as well as the data have not been interpreted against the same specific growth controls. Whether the varying nutrients directly affect the production of toxins or affect the production of toxins indirectly by affecting growth is still unclear. Orr found that the there is a linear relationship between cell division rate and toxin production of *Microcystis.* [9] Downing concluded that microcystin quotas were controlled by variables like nutrient uptake rate other than growth rate, with N having the most significant effect.[25] N and P were also reported to have different effect on MC quota.

Whether excess or limited N, P could increase MC quota or not, or if it's related to growth rate is unclear. [26] N : P ratios were also thought to have an impact on MC synthesis.[27]

Since 2010 RT-qPCR has been used to investigate the effect of nitrogen availability. Phosphate deficiency (N/P 40:1) was found to induce mcyD transcription and microcystin synthesis during the exponential growth phase[28] whereas a slight decrease in mcyD RNA at both higher and lower nitrogen was found at 1-6 day[12]. To date, there is no satisfactory understanding of the mcy transcripts under varying nutrients. mcyD gene expression was found to correlate with MC in cultures.[28] However, Ankita found mcyA expression was not directly correlated with total MC concentrations in the target strains[29]. More comprehensive study needs to be done to elucidate the role of mcy transcripts in MC synthesis as well as their correlations.

2.4 Detection of microcystins

2.4.1 Toxin detection

High throughput analysis methods such as enzyme–linked immunosorbent assay (ELISA), high–performance liquid chromatography (HPLC) and liquid chromatography – mass spectrometry

(LC/MS) were developed to measure toxins based on the knowledge of structures of the most widespread cyanotoxins.[4] However, Toxin analyses are usually time and money consuming and are not able reveal the toxin producers in most cases.

2.4.2 Molecular tool

Molecular detection of toxic cyanobacteria has many advantages over toxin detection.

Quantitative PCR is a real time quantitative technique based on conventional PCR. Fluorescent dyes or fluorescently labeled specific probes are added during the reaction. The fluorescence signal is monitored to accurately quantify the number of starting templates based on the fluorescence signal. qPCR can not only qualitatively detect the presence of cyanobacteria, but also quantitative analyses the toxigenic cyanobacteria.

First, they are easier and cheaper. Conventional PCR or qPCR were developed for a long time. They modulate chain reaction in the cell and can be done qualitatively or quantitatively to determine the presence or quantity of the original DNA copies. Second, they are able to tell the toxin producer. The knowledge of toxic producing gene such as mcy cluster made it possible to target the toxic producing genes. Also, the ITS region has made it possible to distinguish the toxic and nontoxic strains. As long as the appropriate primers are used, we can find out the toxic proportion, quantify the number of toxin-producing *Microcystis* and its proportion in the population. Third, they are more sensitive and can served as early-warning technique. Even when MCs are below the detection limit, such as when pre-bloom seasons, toxigenic cyanobacteria can be detected sensitively and reliably using qPCR. Early warning can help effective prevention of cHABs.

2.4.3 Biomarkers

Biomarkers are biomolecular indicators. DNA can tell “who is there”. When we use the specific functional genes, we could know whether the specific microbe of interest is present and the microbial abundances. But that’s only a potential. From mRNA transcripts, we are able to know the activities. We would investigate the correlation of DNA/RNA with microcystins to figure out whether or not mcyA DNA/RNA could be appropriate biomarker for MCs.

CHAPTER 3: MATERIALS AND METHODS

3.1 Cayuga Lake and Honeoye Lake samples

3.1.1 Sites and sampling

Cayuga Lake is the longest of central New York's glacial Finger Lakes and is the second largest in surface area and volume. It is 64 km long with an average width of 2.7 km. It is approximately 133 m deep at its deepest point. Honeoye Lake is the shallowest of NY State Finger Lakes (9m max depth). Both lakes are popular with fishing and boating events. Nineteen points from Cayuga Lake were sampled during bloom season in 2018. Two samples were received from Honeoye Lake on August 28th and September 11th separately. Four pre-season samples were received on late April 2019 from Cayuga Lake (Detailed information in Table 3). Total chlorophyll-a and MCs were measured by Community Science Institute (CSI). 10-100mL bloom samples were received from CSI on ice. Filtration was processed immediately on a 0.6um microfiber. The volume and weight filtered through was recorded. The filters were stored in -80^{o}C immediately after completion of filtration.

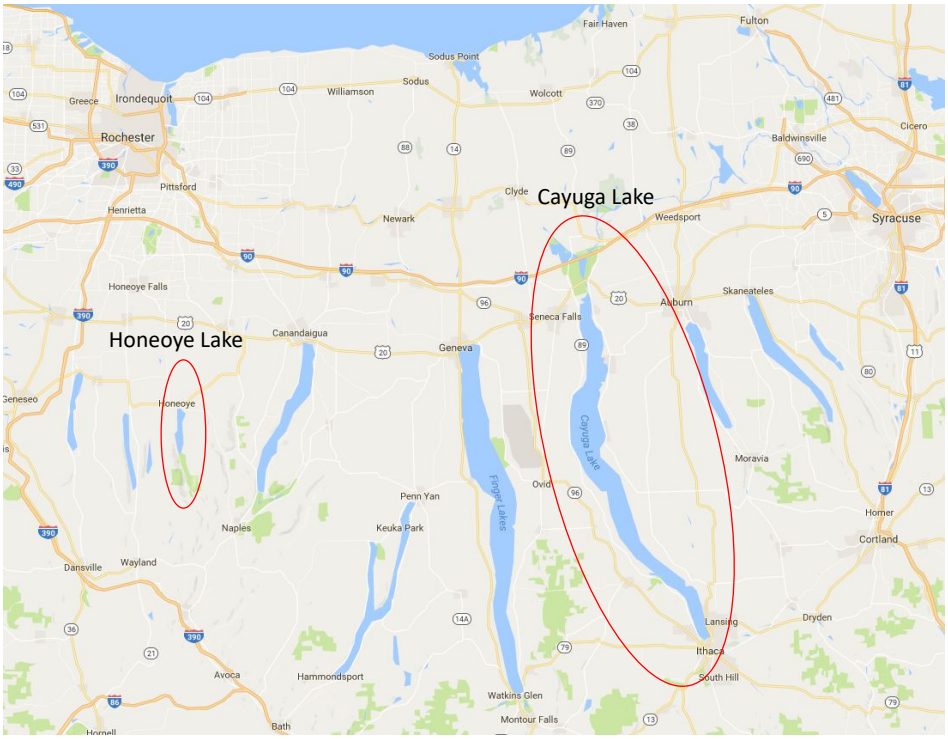

Figure 4: Map of Finger Lakes with Honeoye and Cayuga circled. Source: Google Maps

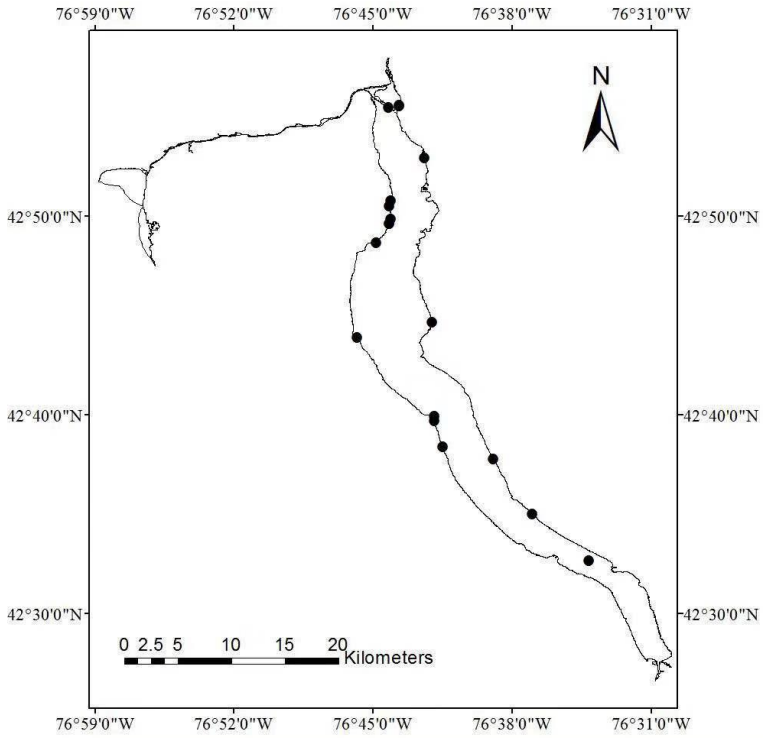

Figure 5: Sampling sites in Cayuga Lake. (Detailed location in Table 1)

Table 1: Samples coordinates

Sample Code	Latitude	Longtitude
18-3400-B4	42.5443	-76.5693
18-3400-B6	42.9255	-76.7285
18-3400-B7	N/A	N/A
18-3401-B1	42.9244	-76.7372
18-3404-B3	42.9261	-76.7284
18-3404-B4	42.882	-76.7075
18-3408-B1	42.744	-76.7005
18-3416-B3	42.6299	-76.6496
18-3418-B4	42.5836	-76.6168
18-3439-B1	42.64	-76.6916
18-3440-B1	42.6617	-76.6988
18-3441-B1	42.6656	-76.6989
18-3444-B3	42.7316	-76.7638
18-3446-B1	42.8109	-76.7479
18-3447-B1	42.827	-76.7366
18-3448-B1	42.827	-76.7366
18-3449-B1	42.8307	-76.7355
18-3451-B1	42.8463	-76.7351
18-3451-B2	42.8417	-76.7364
Honeoye828	4245.064	7730.452
Honeoye911	N/A	N/A
19- CLTG	42.547551	-76.595623
19-CLNE	42.946273	-76.733771
19-CLUS	42.842308	-76.696731
19-CLWE	42.745114	-76.700821

3.1.2 DNA/RNA extraction

Total RNA was extracted from frozen filters using Trizol kit according to the manufacturer's instruction. DNA was further purified using the All prep DNA column (QIAGEN, Valencia, CA) following the associated instruction. Filters were placed in a 2mL tube. 0.3g of 0.5 mm Zirconia-Silica beads (BioSpec Products, Bartlesville, OK) were added to each tube to facilitate cell disruption. The samples were then bead beating for 10 minutes at maximum speed. 4uL of 1E9 luciferase RNA were added into the sample as internal recovery standards for later RNA recovery

calculation. After the phase separation steps, All prep kit was used for DNA purification. Remaining aqueous phase overlying the interphase was removed after Trizol extraction. 0.3mL 100% ethanol per 1 mL of TRIzol™ Reagent used for lysis were added to each sample. Samples were incubated for 3 min. The mixture was then transferred to an All prep DNA spin column. All samples were washed according to the associated Qiagen's protocols and were eluted in a final volume of 100 uL Nuclease-free water. DNA and RNA were quantified with a NanoDrop 1000 spectrophotometer.

3.1.3 PCR and qPCR

DNase and cDNA

Prior to reverse transcription (RT), residual gDNA co-extracted with RNA was destroyed with DNase treatment kit (Promega Inc.). 3 µl 10X RQ1 Reaction Buffer and 1 µl RQ1 DNase were added to each 30 µl RNA sample. Incubate at 37°C for 30 minutes, then 1µl of RQ1 DNase Stop Solution was added to each sample to terminate the reaction. Incubate at 65°C for 10 minutes to inactivate the DNase.

DNA reverse transcription was processed using the iScript™ cDNA Synthesis Kit (Bio-rad Inc.). 5uL RNA sample was added to a total of 20uL reaction volume for cDNA synthesis following iScript protocol. Transcribed cDNA concentration and quality was checked with a NanoDrop spectrophotometer (Nanodrop ND-1000, Thermo Scientific, Waltham, MA).

Conventional PCR was performed on the first 10 samples to check for primer pairs specific for the *Microcystis* mcyA gene. The PCR reaction mixture contained 12.5uL 2X Go Taq, 500nM of each primer in a 25µL reaction volume. All reactions were carried out on a PCR Thermal Cycler using the thermal program: Initial denaturation at 95°C for 5 min, followed by 40 cycles of PCR, each

consisting of 94 ºC for 30 s, 56 ºC for 30 s, and 72 ºC for 60 s. Final extension was at 72 ºC for 10 min.[30] PCR products were run on gel to check results.

The *mcyA* primer set used in this study only targets one of the major microcystin-producing genera: *Microcystis*. [31]

Table 2: Primers used in this study

Gene	Primers/probe	Sequence	Amplicon(bp)	Reference
mcyA	MSF	ATCCAGCAGTTGAGCAAGC		[32]
	MSR-2R	GCCGATGTTTGGCTGTAAAT	190	[31]
16s rRNA	16sF	TCGTCGGCAGCGTCAGATGTGTATAA GAGACAGCCTACGGGNGGCWGCAG		[33]
	16sR	GTCTCGTGGGCTCGGAGATGTGTATA AGAGACAGGACTACHVGGGTATCTAA TCC	450	
mcyA (TaqMan) probe	MISYTM*	TTAAATCGGAAATTATCCCAGAAAAT GCCGT	122	[34]
	MISYF	CGACCGAGGAATTTCAAGCT		
	MISYR	AGTATCCGACCAAGTTACCCAAAC		

Quantitative PCR

Lab based approach on Biorad

Standard curves were generated using purified recombinant plasmid DNA containing *Microcystis* mcyA gene inserts. Briefly, tenfold serial dilutions of plasmid DNA samples were performed to obtain solutions with the respective gene copy numbers ranging from 10^8 to 10^2 per microliter. Subsequently, qPCR was performed on triplicate dilutions using Bio-rad iCycler qPCR System.

Each 25 µL qPCR reaction mixture contained 2µL DNA template, 12.5uL 2× iQ SYBR Green Supermix (Bio-Rad, US), mcyA forward (500nM) and reverse (500nM) primers. Thermal cycling was conducted on an iCycler IQ (Bio-Rad) with the following protocols: initial activation at 95°C for 5min, followed by 40 cycles consisting of denaturation at 95°C for 10s and combined annealing/extension at 60°C for 30s. Melt curve analyses were conducted on all products to check for nonspecific amplification.

Biomeme approach

The Biomeme two3™ (Product code 300003) is a mobile thermocycler for real-time linear probe polymerase chain reaction (PCR), isothermal and RT-PCR analysis of DNA or RNA could be investigated to develop a rapid, simple, low-cost detection method of toxin-producing cyanobacteria. It's easy to use, portable, handheld and could be done in the field/ in-situ.

The Biomeme two3 ™ has two channels (FAM and Cy5) and three wells so duplex reactions can be run for three samples simultaneously. We can include IPC strip to check for inhibition. An updated version can be worked using cocktail to solve inhibition.

3.1.4 Sequencing and genomic work

Illumina 16S rRNA amplicon sequencing was used to characterize the bacterial community as a whole – thereby enabling profiling of community composition of cyanobacteria and bacteria living on/among the bloom colonies. 16S MiSeq sequencing was sent out for test, data were processed using QIIME2, and further analysis was via PhyloSeq data visualization using R and Excel.

For Qiime2 (https://qiime2.org/), demultiplexed sequences were denoised and clustered into amplicon sequence variants (ASVs) using DADA2[35] with a max EE value of 12. ASVs were

annotated for taxonomy against the SILVA 132 99% OTUs from 515F/806R region of sequences database (https://www.arb-silva.de/).

3.2 Microcystis PCC7806 Nutrient experiments

3.2.1 Experiment design and growth measurements

Microcystis aeruginosa PCC7806 obtained from Pasteur Culture Collection (Paris, France) was grown in BG-11 medium in batch culture. The pH of the media was adjusted to before 7.5 Autoclaving.

For the comparative experiments in this study, different nitrate and phosphate availability conditions were set under three different conditions (Table 1). Growth was monitored every two days determining the optical density at 600 nm and chlorophyll a was also quantified at 625nm on Tecan Infinite 200 PRO (TECAN, Switzerland). *Microcystis* were inoculated 10% Vol: Vol using stationary phase cells to 100mL of culture media in 250mL Erlenmeyer flasks with Silicone Sponge Closures (VWR). Cultures were incubated at 25^{o}C ± 1^{o}C and agitated on a rotary shaker at 66 rpm. The flasks were maintained under white fluorescent light of 10 µmol of photons m^{-2} s^{-1} with a light: dark cycle of 12:12h. Before transferring to new conditions, 60mL of cultures were centrifuged at 4000rpm for 10 minutes and washed in DI water for three times.

Table 3: Nutrients experiment setting

	N(mg/L)	P(mg/L)	N/P(mass)
Balanced growth	31	6.2	5
N excess	155	6.2	25
P excess	31	31	1
BG11	247.1	5.4	45.5

3.2.2 Nutrient measurements

Dissolved nutrients were measured every two days in 1mL aliquots of culture after centrifuge 8000rpm for 5 min to remove cells. The supernatant was stored in -20^{o}C until nutrient measurements. A rapid, simple spectrophotometric method was used for simultaneous detection of nitrate and nitrite with measure of absorbance of at 540nm. [36]Phosphorus was determined using spectrophotometric method measured at 880nm.

3.2.3 DNA/RNA extraction

0.5 mL aliquots of fresh culture were centrifuged at 10000rpm for 10 min to remove supernatant, and pellets were stored in 1mL Trizol reagent at −20 °C until DNA/RNA extraction. DNA/RNA extraction procedures were the same as 3.1.2.

3.2.4 qPCR and Microcystin extraction

Quantitative PCR procedure was the same as 3.1.3.

Aliquots of 2-10mL of cells were pelleted and stored at −20 °C until microcystin extraction. Pellets were placed in 2mL microcentrifuge tubes, allowed to thaw at room temperature for 5min freeze and thaw for three minutes. Freeze at -70^{o}C for 10 min, then thaw at 37^{o}C for 5 min. Repeat 3 times and subsequently centrifuged at 10000 g for 10 min. The clarified supernatant was transferred into new microcentrifuge tubes and diluted with double distilled water 1000-fold prior to ELISA analyses. All reagents and samples were placed at room temperature for 30 minutes. 100 µL of negative control, calibrator, and sample was added to respective wells, then incubate for 30 minutes. After that, add 100 µL of microcystins-enzyme conjugate to each well. After another incubation of 30 minutes, all wells were washed step with double deionized water for five times. Then, 100 µL of substrate was added to each well before another incubation of 30 min at room temperature. Finally, 100 µL of stop solution was added to each well. The obtained color and its related absorbance, where toxin concentration is inversely proportional to color development, were

read at 450 nm immediately. MCs values were calculated using a standard curve (0.1–1.6 ppb). Microcystin concentration was spectrophotometrically determined using a 96 well EnviroGard® microcystin plate kit (Strategic Diagnostics, Newark, DE, USA) and Tecan Infinite 200 PRO (TECAN, Switzerland) reader. The EnviroGard® ELISA microtiter plates utilized polyclonal antibodies that allowed for detection of microcystin-LR, -RR, and -YR. All toxin concentrations are reported as MC-LR equivalents, with the assay having a limit of detection of 0.1µg L^{-1}

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Characterization of cyanobacterial communities in two Finger lakes

4.1.1 Microbial community profiles in Cayuga Lake and Honeoye Lake

Nineteen samples were collected from Cayuga Lake at different sites (Figure 4) where there were suspicious blooms, on 6 different days of 2018 August to September. Two samples were collected from Honeoye Lake where there were also visible blooms on August 28th and September 11th, 2018, respectively. Four samples were taken on April 25th, 2019, with some samples having overlapping locations with the previous samples (Table 1).

One of the goals of this study was to determine the microbial community profiles in two of the Finger Lakes, Cayuga Lake (a local lake near Cornell) and Honeoye Lake (91km away from Cayuga Lake). A thorough knowledge of the community structure would help understand the ecology of local microbial groups during HAB events as well as identify the potential toxin producing cyanobacteria. Thus, prokaryotic 16S ribosomal RNA gene (16S rRNA) amplicon sequencing was used to analyze the community profile information in some samples collected during late summer 2018.

1132079 total reads were retrieved across all 28 samples. In Cayuga Lake, across the whole dataset the majority of reads were from the phylum cyanobacteria (59%), with Bacteroidetes being the second most abundant phylum (Table 4). Proteobacteria were also detected at many sites, and a small portion of reads were from the Planctomycetes. An interesting note about the Bacteroidetes group is, the most abundant genus *Flavobacteria* in this group were recently found to be putative photoheterotrophic [37]. In addition, some species of Bacteroidetes were found to have an increase in biomass following an increase of input of organic matter possibly resulting from the death of cyanobacteria.[38] Among cyanobacteria group, *Microcystis*, was found to be the major genus

(82%) in Cayuga Lake, which is in agreement with the microscopic information notes by CSI staff (detailed information in S1). *Pseudanabaena* was the second most abundant genus (12%). *Aphanizomenon*(2.25%) were also found at a few sites. According to a list from USGS, the most abundant four cyanobacteria genera were all capable of producing MCs. Overall, in Cayuga Lake, *Microcystis* is the most abundant cyanobacteria species as well as the major MCs producing genus, as suggested in other lakes worldwide.

Table 4: Top 4 phyla ratio in Cayuga Lake and Honeoye Lake

	Cayuga	**Honeoye**	**Cayuga RNA**
Cyanobacteria	59%	45%	83%
(Cyano range)	0-82%	15-58%	62-97%
Bacteroidetes	17%	16%	4%
Proteobacteria	17%	10%	11%
Planctomycetes	1%	1%	2%

Table 5: Top 4 genera ratio among cyanobacteria

	Cayuga	**Honeoye**	**Cayuga RNA**
Microcystis	82%	20%	91%
(Microcystis range)	0-100%	19-20%	76-100%
Pseudanabaena	12%	0.30%	8.40%
Synechocystis	0.04%	48.25%	0.00%
Aphanizomenon	2.25%	0.00%	0.22%

Honeoye Lake had a much different community structure. It had a more diversified bacterial group. Comparing the data from 2018 August 28th with September 11th, cyanobacteria relative abundance increased from 15% to 58% (Table 4), while Bacteroidetes reads dropped from 29% to 9%. Among cyanobacteria phylum, Honeoye Lake had more *Synechocystis* (Table 5). *Microcystis* percentages among cyanobacteria phylum reads were about 20% on both dates while *Synechocystis* raised from

31% to 50%. It's not surprising Honeoye Lake had a much different community structure than Cayuga Lake, since they differ a lot in lake size, depth etc. The lower presence of *Microcystis* may explain the observation of lower MCs in Honeoye vs Cayuga samples (see MC results in Figure 11).

When compared Cayuga RNA samples with Cayuga DNA samples, we can see RNA from cyanobacteria was more active than RNA from other phyla. The dominance of cyanobacteria in the RNA-based 16s amplicon sequencing was even stronger than in the DNA with 62-97% of RNA-based reads from the cyanobacteria phylum. Similarly, among cyanobacteria phylum, RNA from *Microcystis* was more active than RNA from other genera.

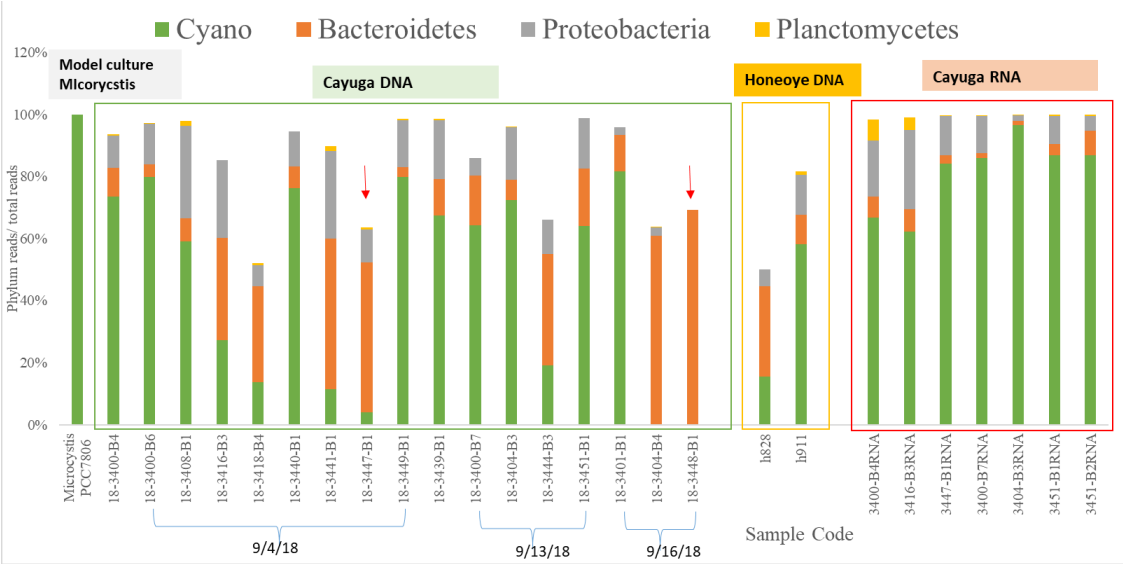

Figure 6: Relative abundances of the most abundant four phyla at different sites from Cayuga Lake ("18-XXXX") and Honeoye Lake (h818, h911) summer 2018. (PCC is the pure culture *Microcystis* PCC 7806 used in lab experiments).

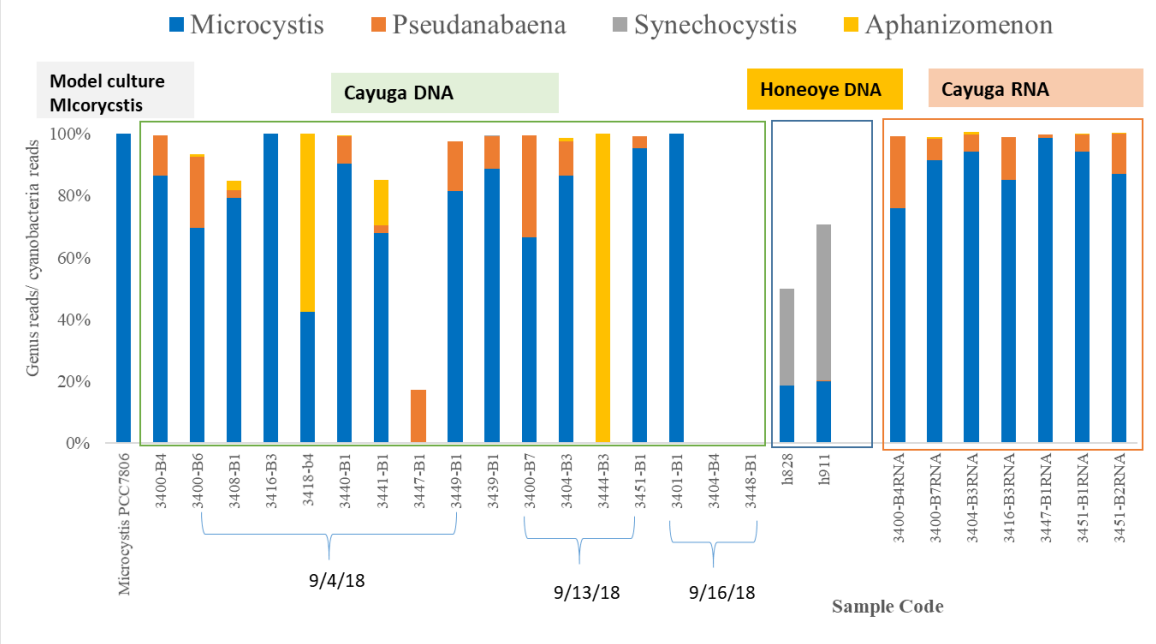

Figure 7: Relative abundances of the most abundant four genera in the Cyanobacteria Phylum at different sites from Cayuga and Honeoye samples in August/September of 2018.

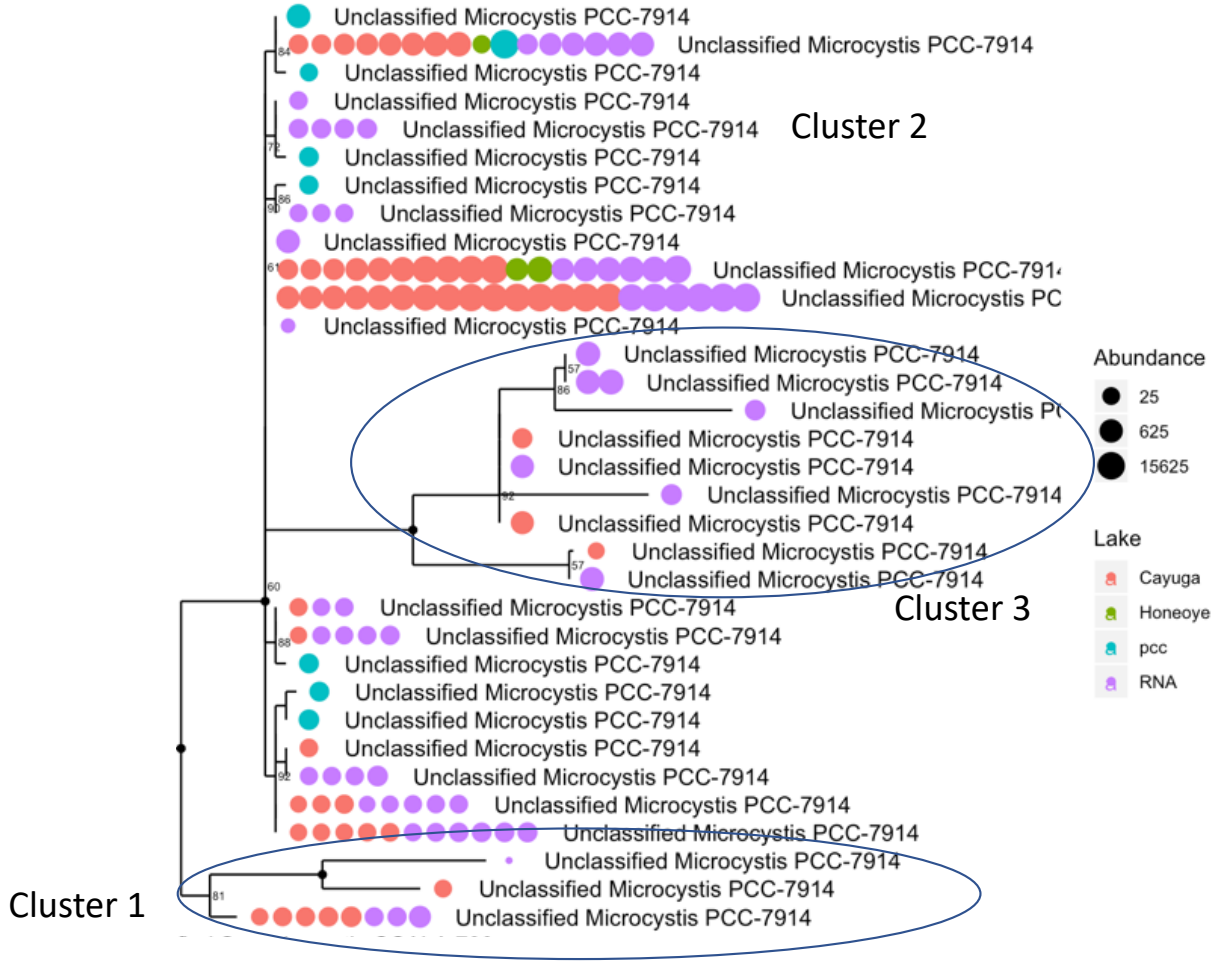

Figure 8: Phylogenetic tree of different Cyanobacteria Species from Cayuga and Honeoye Lake samples 2018 summer color coded by three clusters according to PCoA plot. With each dot represent one sample and size represent each relative abundance.

According to phylogenetic tree of *Microcystis*, more than one type of *Microcystis* were found as distinct 16S rRNA sequenced (Figure 8). There were basically three different clusters according to the sequence divergence distance on the tree. The most ubiquitous cluster (cluster 2) was within the same group as the model strain *Microcystis* aeruginosa PCC 7806, which is the toxin producing model strain used in culture experiments presented later. Although from 16S rRNA gene sequencing we cannot tell whether each strain is toxic or not, we could infer that some of them are toxic since we have detected toxin synthesis genes mcyA (Table 4) and mcyE (S2) in all sites. We

can also tell that Cayuga and Honeoye had different *Microcystis* groups. Among each cluster in Figure 8, each branch may represent different Actual Sequence Variants (ASVs) as proxies for species/strains, but the quality of reading library was too low to conclude whether some of the ASVs were spurious rather than true strains. Further work needs better understanding at the strain level of *Microcystis* and determine which strains/species are producing toxins.

Three different clusters were found (Group 1,2,3) according to Principal Coordinates Analysis (PCoA) (Figure 9) of samples 16s profiles. Group 2 cluster together probably because they all had low total reads (Figure 10). However, the reason why group 2 had low total reads was unknown. Also, current data was not able to explain why other two groups clustered. More metadata was needed to understand what is driving the similarity in those clusters.

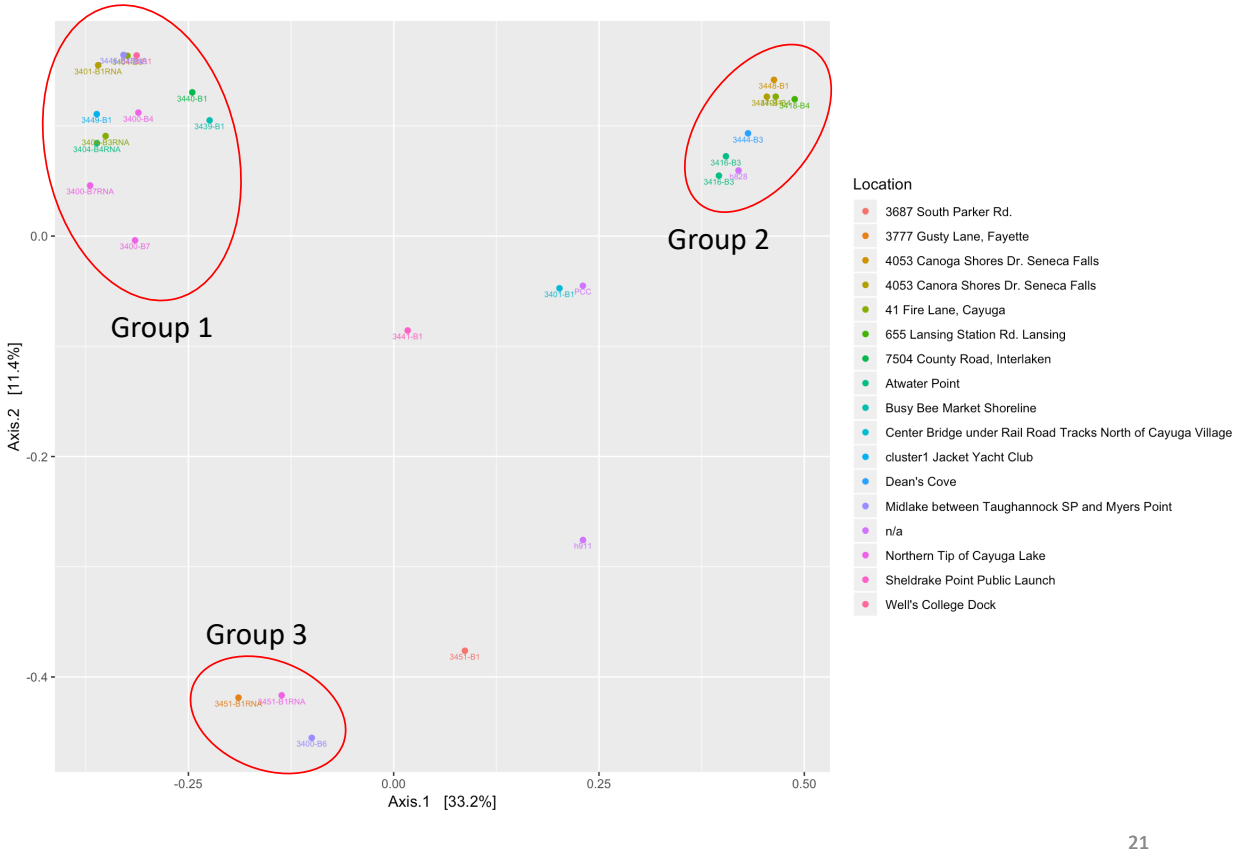

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Figure 9: PCoA coordination of different samples from Cayuga and Honeoye Lake 2018 summer, color coded by specific location ID.

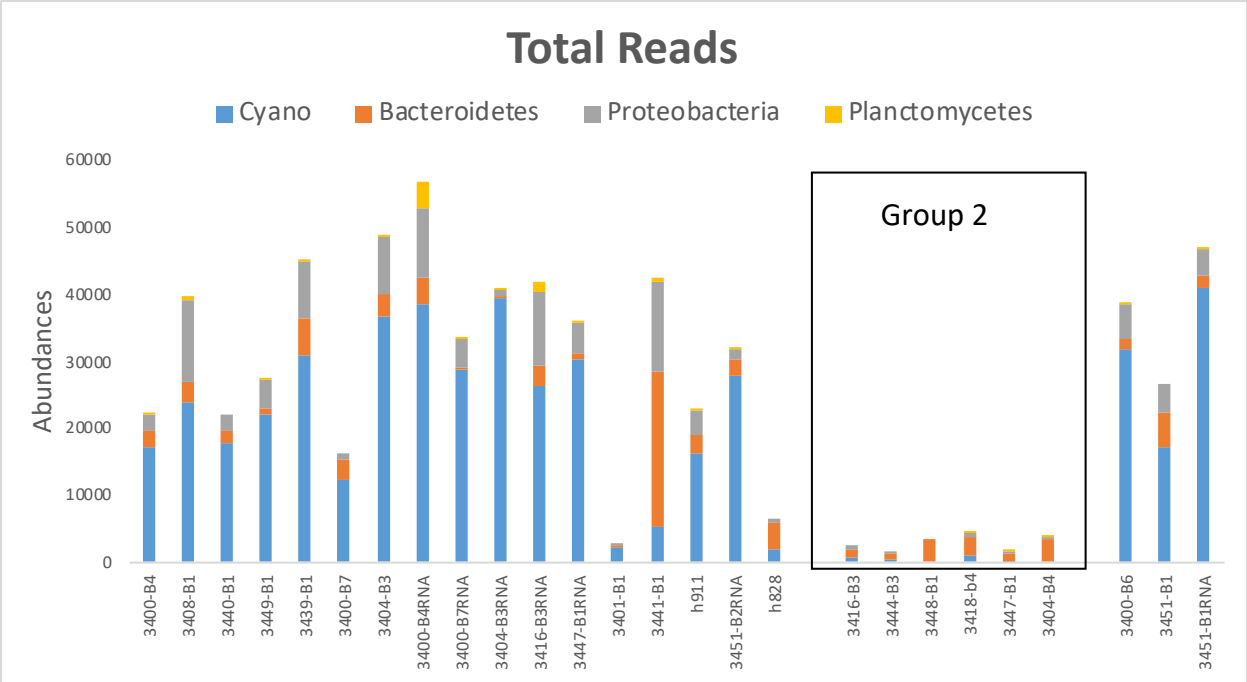

Figure 10: Total reads of 16s amplicon sequencing of different samples

4.1.2 Microcystin and mcyA DNA/RNA levels

Microcystin concentrations of bloom samples from Cayuga Lake in August and September 2018 were generally confirmed with high toxins (>20ppb). For different sites, the concentrations varied a lot. The lowest concentration was below 0.3 µg/L whereas the highest concentration reached 2500 µg/L being thousands folds higher than the limit which pose public health risk. Two samples from Honeoye had low MCs concentrations with 0.35 and 1.4 µg/L respectively, both being low. *Microcystis* mcyA genes were detected in all samples. mcyA DNA was all quantifiable via Biorad qPCR assay while cDNA levels from RNA were below detection limit at some sites. Cayuga Lake mcyA DNA copies abundances ranged from 1.41E3 to 1.24E8 copies/mL with an average of 9.73E+06 copies/mL, while Honeoye Lake had an average of 6.41E+04 copies/mL. As we talked in previous section, Honeoye Lake had less *Microcystis* abundance compared to other cyanobacteria which resulted in less toxicity compared to Cayuga Lake. Four more pre-season non-bloom samples were taken from Cayuga Lake in April 2019. Their DNA copies were much

lower than the summer 2018 samples and ranged from 2.6E01 – 2.17E04, indicating presence of toxic *Microcystis*. Though at the pre-season point, those sites had a lower toxic gene abundance, microbes there still may have the potential to massively reproduce and produce a large amount of toxins when environment conditions become suitable.

Table 6: mcyA gene copy and RNA transcripts levels of summer 2018 Finger Lakes samples from Cayuga ("18-34XX") and Honeoye and Cayuga 2019 pre-season blooms.

Sample Code	DNA copies/mL	RNA copies/mL	MCs(μg/L)	Chl-a(μg/L)
18-3400-B4	2.28E+06	N/A	N/A	25.1
18-3400-B6	1.93E+07	5.65E+08	2533.2	7766
18-3400-B7	3.49E+05	4.94E+08	214.7	4114
18-3401-B1	1.24E+08	4.90E+07	52	444
18-3404-B3	4.35E+06	1.85E+08	41	693
18-3404-B4	6.68E+03	1.89E+08	272.03	1540
18-3408-B1	1.35E+07	N/A	12.25	45.8
18-3416-B3	1.50E+05	1.01E+11	374	5434
18-3418-B4	4.57E+03	N/A	<0.3	389
18-3439-B1	5.34E+06	N/A	310	1188
18-3440-B1	2.71E+05	2.63E+07	54.8	785
18-3441-B1	2.54E+06	N/A	>5	1093
18-3444-B3	2.11E+04	b.d.	281.7	2406
18-3446-B1	6.16E+03	1.20E+10	498.25	3410
18-3447-B1	2.58E+04	2.60E+07	389	909
18-3448-B1	1.41E+03	7.57E+09	1604	6314
18-3449-B1	4.38E+06	1.94E+12	101.52	365

18-3451-B1	8.13E+06	3.05E+08	40.28	869
18-3451-B2	1.46E+05	8.26E+07	55.1	1192
Honeoye828	9.35E+04	b.d.	0.35	35~40
Honeoye911	3.47E+04	4.00E+07	1.4	37
19- CLTG	2.14E+02	b.d.	<0.3	N/A
19-CLNE	2.17E+04	b.d.	<0.3	1.81
19-CLUS	1.96E+04	b.d.	<0.3	N/A
19-CLWE	2.60E+01	b.d.	<0.3	7.7

b.d. indicates below detection. MC (microcystin levels) and Chlorophyll-a readings were performed by the Community Science Institute which is certified for MC readings.

4.1.3 Biomarker and biomass correlation with MCs

Table 7: Correlations between mcyA gene expression and copies, chl-a and microcystin concentration data from Cayuga Lake 2018 and 2019 samples.

Cayuga 2018 & 2019 samples (with below detection limit samples)			
	log chl-a(μg/L)	log DNA (copies/mL)	log RNA (copies/mL)
log DNA (copies/mL)	-0.143(n=21)		
log RNA (copies/mL)	0.698**(n=15)	0.505*(n=17)	
log MC (μg/L)	0.857**(n=19)	-0.421(n=21)	0.834**(n=17)

**P < 0.01, *P<0.05; otherwise not statistically significant. (n is the number of observations)

In order to investigate whether DNA or RNA could be good bimolecular indicator of MCs, Pearson correlation coefficients were calculated between mcyA copies, mcyA transcripts, microcystins as well as chlorophyll-a using log10 transformed data. The results are presented in Table 6.

Total chlorophyll-a concentrations ($r=0.857$, $p<0.01$) and RNA levels ($r=0.834$, $p<0.01$) were significantly correlated with MCs, whereas *Microcystis* mcyA DNA copies had a poor correlation with MCs.

Chl-a concentration can serve as a good indicator of microcystin concentration. As chl-a is only present in living cells, which might indicate how cells are actively expressing toxins. Other papers

also found chl-a to have a good correlation with MCs, suggesting chl-a could be a good indicator when bloom actually happens[39], telling us some information about toxicity. Compared to *Microcystis* mcyA gene copies, chl-a can also account for different species that are able to produce toxin where as mcyA in our study only target *Microcystis*. Zamankhan found the measured biological toxin concentration (MCs) to be strongly correlated with the cyanobacterial bloom activity (CI) estimated by satellite image analysis. The phycocyanin concentration also had a strong correlation with CI, implying that measuring an easy-to-detect proxy parameter in-situ and in real-time is effective for monitoring cHABs. [40]

However, chl-a tells little information about which species are producing toxin and lacks the ability to pre-warning for possible blooms. Moreover, for the blooms that are dominant by nontoxic cyanobacteria, chl-a might not be a good indicator for the toxicity. Thus, more sensitive and rapid measurement at biomolecular level would be promising not only because of its ability to target toxic strains but also for early-warning.

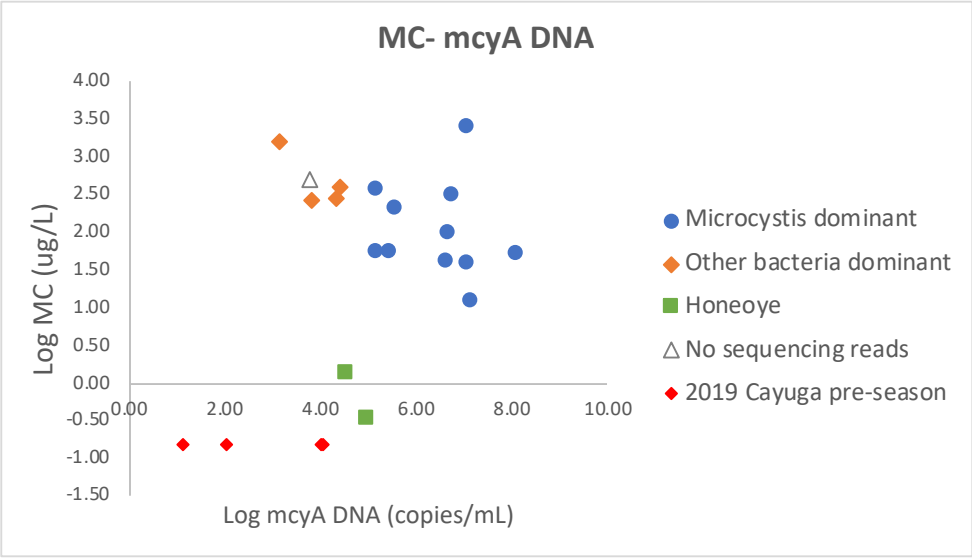

Figure 11: mcyA DNA vs MC level grouped by dominant genus found in Illumina 16 S rRNA gene libraries from extracted DNA (with information from Figure 7)

There was not a significant correlation between mcyA DNA and MCs in Cayuga 2018 and 2019 samples. When combined with the results from 16s sequencing, we grouped the mcyA DNA data points from Cayuga Lake by those dominated by *Microcystis* or not (Figure 11). It was interesting to find those samples dominated by *Microcystis* tended to have a different trend with MCs than those dominated by other bacteria. That could be because we chose primers that targeted *Microcystis'* mcyA genes specifically, which could lead to underestimation of the total mcyA genes from other genera such as *Aphanizomenon* and *Pseudanabaena*. We could imagine that, if we choose or redesigned more general primers, those data points from samples dominated by non-*Microcystis* genera (orange points in Figure 11) would shift to the right on the graph, which could drastically change the overall trend. Also, we haven't test inhibition for those samples. If inhibition is present, those data points would shift to the right on the plot in Figure 11. Further study should apply more general primers and qPCR formulations that overcome inhibition problem to get a better correlation of mcyA gene and MCs.

However, in field samples, it is also possible for cyanobacteria to contain the target gene of interest, but be absence of one or more of the vital *mcy* genes via gene deletion, recombination, or transformation or due to gene disruption and inactivation by transposons or phage [29]. More import to note is that even with full sets of mcy genes, they may not have the pathway transcribed into mRNA. So, RNA is promising in providing us with such information.

The four-pre-season MC levels were all below detection limits, not surprisingly, their RNA transcripts levels were also below detection limits. If we included the four pre-season below detection (b.d.) samples using the half detection limit as a placeholder value for the "censored" true value, an obvious trend was observed, which making RNA significantly correlated with MC ($r=0.834$, $p<0.01$). The one Honeoye 2018 sample with detectable RNA was off the linear trend

for the Cayuga samples in Figure 12, which could be due to the fact that it had a different community structure. While chl-a and DNA might both be good indicators of MCs when the bloom was dominated by toxic cyanobacteria, RNA would be good proxy for MCs even the bloom is dominated by non-toxic cyanobacteria.

There was a possibly-significant limitation during our RNA sampling and processing procedure since RNA is easy to be degraded in the environment. Some samples were sent to us 2-4 days later after they have been sampled. The holding times and conditions (refrigerated or on ice) varied with some samples being processed for RNA within 6 hours and others for 4 days. This might cause shifts in RNA that didn't reflect levels in the original sample. A better way to preserve the RNA samples and/or processed promptly is needed in the future. Nonetheless, even with this potential limitation, we found a promising correlation between mcyA transcript levels and MC levels.

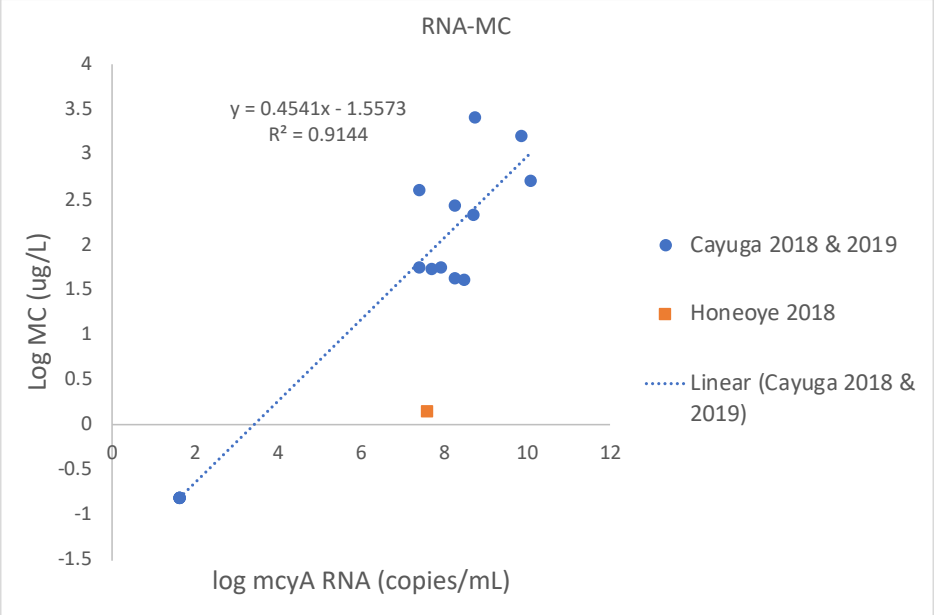

Figure 12: RNA copies versus MCs with varying different holding time (The Below detection place contain 4 data points)

Biomeme qPCR inhibition testing with mcyA quantification

Figure 13: Biomeme qPCR workflow

For DNA, we have some samples checked for qPCR inhibition (Figure 15) on a handheld qPCR device, Biomeme two3 (Figure 13). The Biomeme two3 device has three wells for each run at a time. While each well has two channels, on for targeting sample fluorescence of the mcyA gene TaqMan assay, another for targeting the internal positive control (IPC) added to the reaction to test for inhibition. If for all three wells, the IPC strips came out at a similar position, which indicates no inhibition. For one field sample tested on this device (Figure 14), its corresponding IPC signal came out much later than the other two culture samples run at the same time, which indicated inhibition present in that sample. For environmental samples, inhibitors might be present for qPCR amplification, which could underestimate the original DNA and/or RNA copies in the sample. For the seven DNA samples tested, three showed complete inhibition while 4 showed little inhibition via the IPC test. We are now testing Biomeme two3 device with cocktail to solve the inhibition problem to make qPCR more accurate.

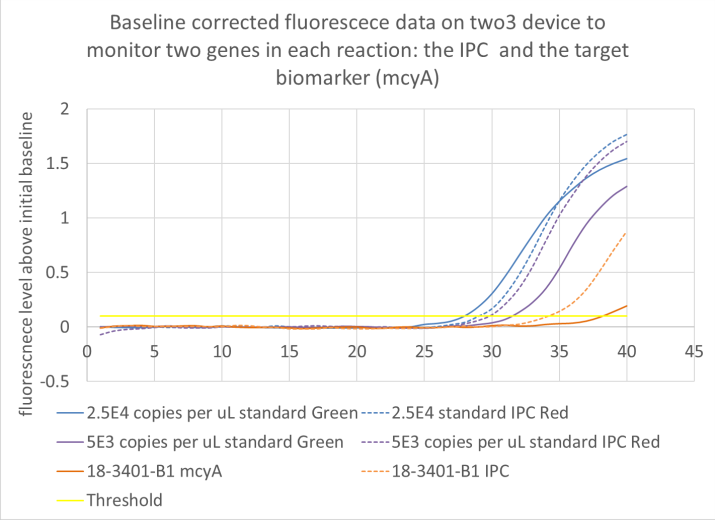

Figure 14: Comparison of qPCR amplification cycles for one inhibited field samples and two uninhibited culture samples on Biomeme two3 device.

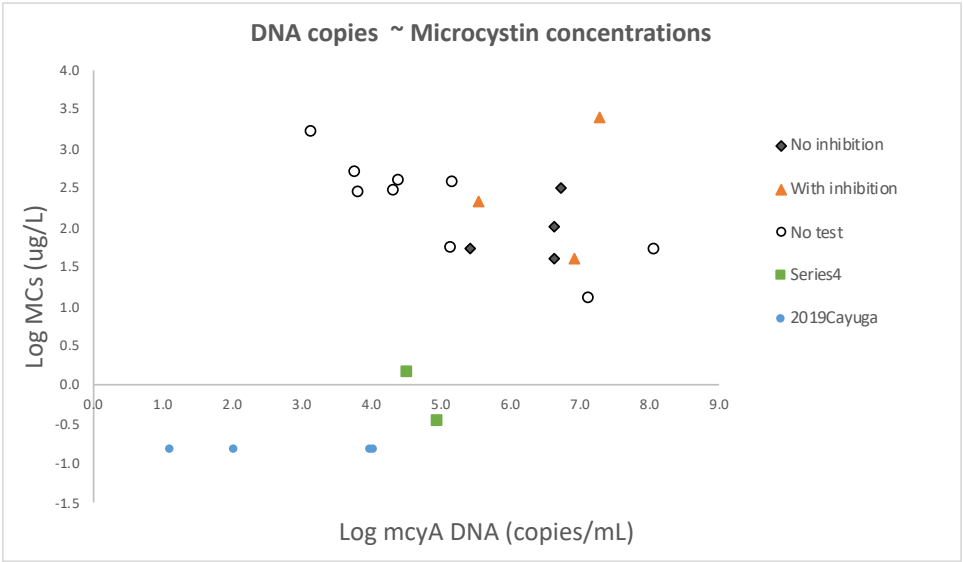

Figure 15: log mcyA DNA copies showing test with inhibition.

Besides an inhibition test, Biomeme two3 device was also tested for the ability to quantify the original mcyA gene copies. A standard curve (Figure 16) with R^2=0.9924 was observed to be used for later quantification. The whole procedure took less than 1 hour, which is much faster than the conventional Biorad lab machine. The potable, accurate and fast features of this device making it possible for field in-situ testing.

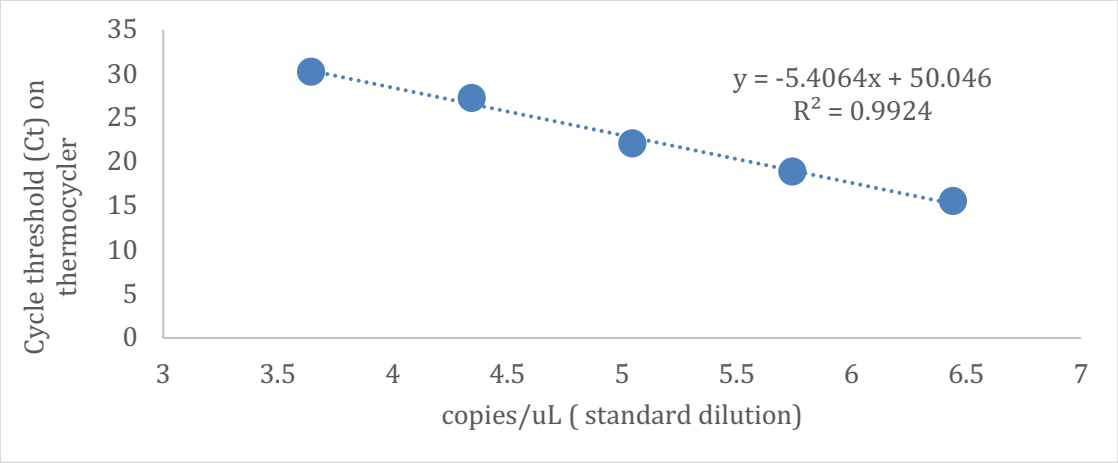

Figure 16: Standard curve on Biomeme two3 using *Microcystis* culture mcyA genes with series dilutions

4.2 PCC7806 culture experiments

4.2.1 Growth in different N:P concentrations

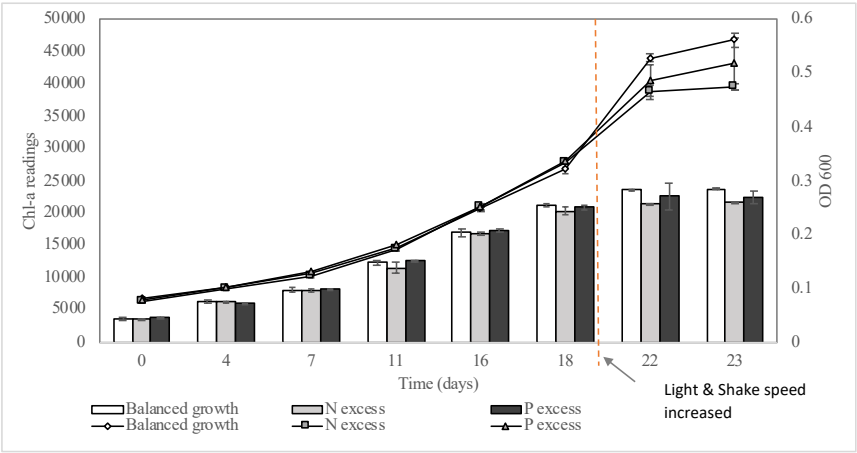

Figure 17: Growth curve of M. aeruginosa PCC7806 cells under different N:P ratios. The actual concentration of N and P as table x indicates. Estimation of growth using chl-a (left, line) and optical density (right, bar).

Cellular chl-a was measured using fluorescence at an excitation wavelength of 614 nm and measured at 625 nm. Similar trend was observed in OD 600 readings (Figure 17).

Under excess nutrients conditions, increase in growth was not found, which might indicate cell growth limited by light or CO_2. That could explain why no trend was found either of DNA or RNA

copies under different nutrient ratios. Though we expected divergence as cultures run out of one or both nutrients, they probably didn't reach stationary phase yet.

On day 21 light was increased from 10 to 20 µmol of photons m^{-2} s^{-1} while shaking speed was changed from 66rpm to 80 rpm to decrease the time to reach a stationary phase.

4.2.2 *Microcystis* mcyA transcriptional and MC producing response to nutrients ratio

PCC7806 as a model toxic *Microcystis* strain, was found to be always producing MCs under different conditions.

Over time, we can see an increase in mcyA gene copies, RNA copies and MCs. However, no trend was found comparing different nutrient conditions. A later time point needs to be sampled to get information of how nutrient limitation would affect mcyA expression or MC producing under different nutrient conditions.

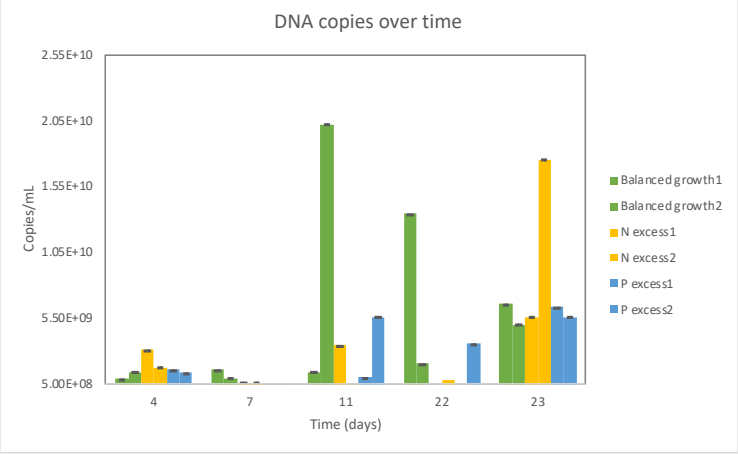

Figure 18: DNA copies over time

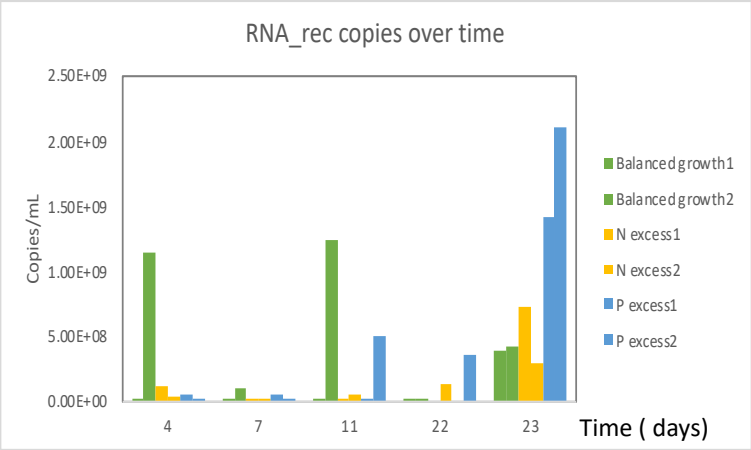

Figure 19: RNA copies over time

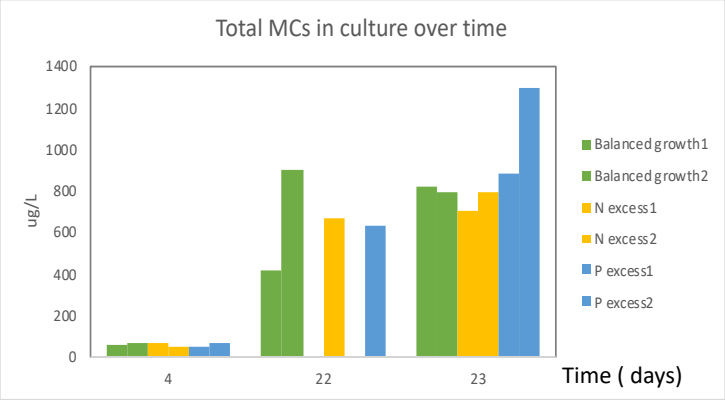

Figure 20: Total MCs over time

Normalizing RNA to gene copies (Figure 21) also increased with time suggesting RNA become more active in the later days.

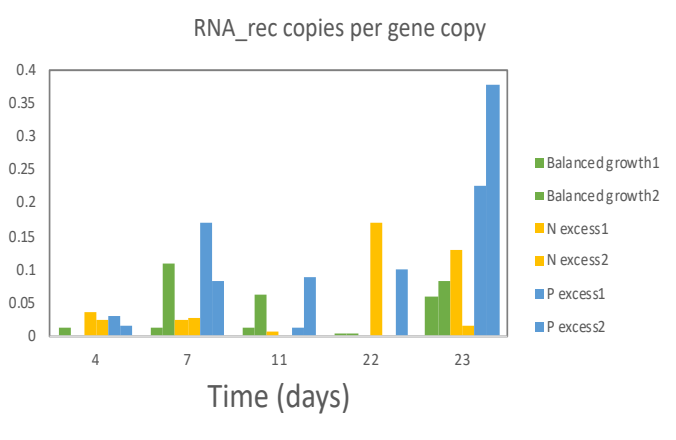

Figure 21: RNA/gene copy over time

4.2.3 Correlation analyses in lab cultures

A major goal of this experiment was to investigate the correlation of mcyA DNA and RNA copies with MCs in a more controlled lab condition. Pearson correlation coefficients were calculated between mcyA copies, transcripts, chlorophyll-a. and MCs. The results are presented in table x.

Table 8: Correlations between *Microcystis* mcyA transcripts and copies, and MC concentration data from mono culture (PCC 7806) experiments.

	log chl-a	log DNA (copies/mL)	log RNA (copies/mL)
Log DNA (copies/mL)	0.619*		
log RNA (copies/mL)	0.350	0.387	
log MC (µg/L)	0.982**	0.589*	0.426*

(**p<0.01, *p<0.05)

Chl-a was found to be best correlated with MCs, which indicates chl-a can be a good indicator of MCs at least in toxic strain dominant conditions. RNA transcript levels were also found to have a significant correlation with MCs, which supported our hypothesis that in a more controlled condition, with proper sample processing procedure, RNA levels could be good indicators of MCs. DNA was found to have a correlation with chl-a and with MCs. The monoculture only contains one toxic producing strain, it is not hard to understand mcyA DNA copies were correlated with MCs.

CHAPTER 5: CONCLUSIONS AND FUTURE WORK

5.1 Conclusions

1. DNA pools from Cayuga Lake indicated bacteria communities were dominant by cyanobacteria (59%) with *Microcystis* being the most abundant genus (82% of cyanobacteria) and the major MC producer. Different strains of *Microcystis* were detected. Honeoye Lake had a different community structure. The results from RNA showed that cyanobacteria were very active.

2. mcyA genes were detected in all field samples. The correlation analysis chl-a was the best indicator of MCs at least in toxic strain dominant environment. In Cayuga 2018 bloom samples (August/September) and 2019 pre-bloom samples (April), only mcyA RNA levels showed significant positive correlations with MC concentrations, while mcyA DNA levels did not.

3. A rapid (<1 hour) and potable qPCR/RT-qPCR device Biomeme two3 would be promising in further in-situ monitoring of toxic cyanobacteria genes. It includes an internal positive control (IPC) to test for qPCR inhibition which would improve the accuracy of DNA quantification.

4.During different growth phases *Microcystis aeruginosa* PCC7806 always produced MCs under different N/P ratio condition. No trend was found under different nutrients conditions regarding MCs production. However, RNA levels did correlate with MC levels.

5.2 Future work

1. Identify different *Microcystis* at strain/species level to discern the toxin-producing strains. Further sequencing analysis to better understand the community structures and ecologies.

2. Measure more metadata such as N, P to understand the environmental factor impacts.

3. Reworking qPCR formulations to counter inhibition issue. Design more general primers to get better DNA-MC correlations.

4. Further sampling get more baseline samples (nonbloom times). Better communication to get sample promptly or include RNA preservation protocol during sampling.

5. Continue sampling of lab culture PCC7806, to compare MC production as cultures run out of N, P or both simultaneously.

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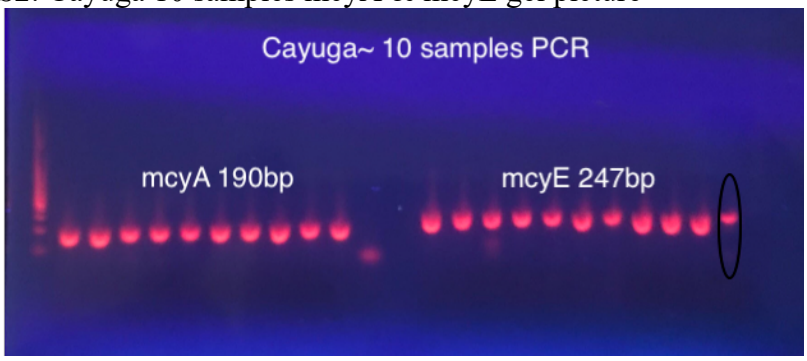
APPENDIX

S1: Link for overall 2018 sample data

https://docs.google.com/spreadsheets/d/1SDPwBluh-a81EUHZWRJaW4vfN1Z5zlnzNJIO8ujwOCI/edit#gid=1878265517

S2: Cayuga 10 samples mcyA & mcyE gel picture



S3: BG 11 medium:

Stock 1:

Na_2MG EDTA	0.1g/liter
Ferric ammonium citrate	0.6g/liter
Citric acid $\cdot$ $1H_2O$	0.6g/liter
$CaCl_2 \cdot 2H_2O$	3.6g/liter

Filter sterilize into a sterile bottle or autoclave, add 10mL after others mixed

Stock 2:

$MgSO_4 \cdot 7H_2O$	7.5g/liter

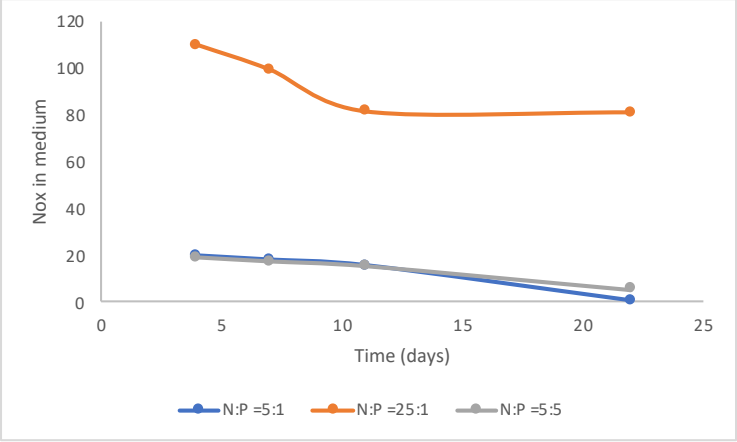
Stock 3 (Microelements):

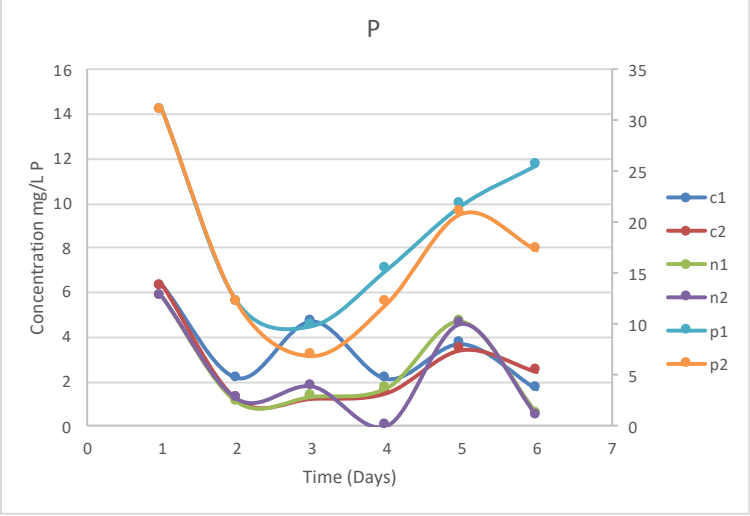
H_3BO_3	2.86g/liter
$MnCl_2 \cdot 4H_2O$	1.81g/liter
$ZnSO_4 \cdot 7H_2O$	0.222g/liter
$CuSO_4 \cdot 5H_2O$	0.079g/liter
$COCl_2 \cdot 6H_2O$	0.050g/liter
$NaMoO_4 \cdot 2H_2O$	0.391g/liter

or MoO_4 (85%)	0.018g/liter

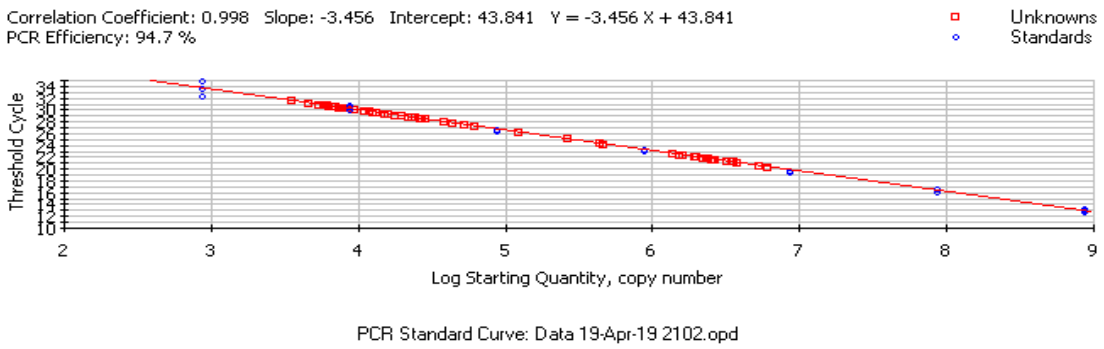
Stock Solution	Per Liter of medium
Stock 1	10 ml
Stock 2	10 ml
Stock 3	1 ml
Na_2CO_3	0.02g
$NaNO_3$	1.5g
K_2HPO_4	40mg

S4: Nutrients left in culture.

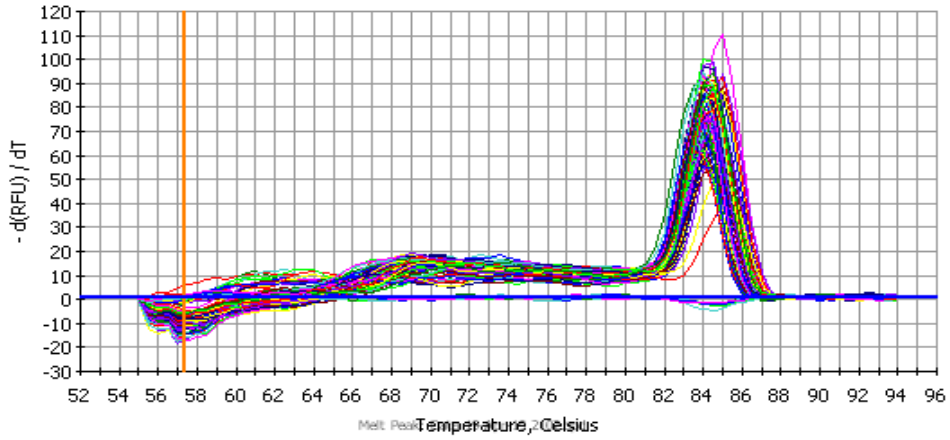




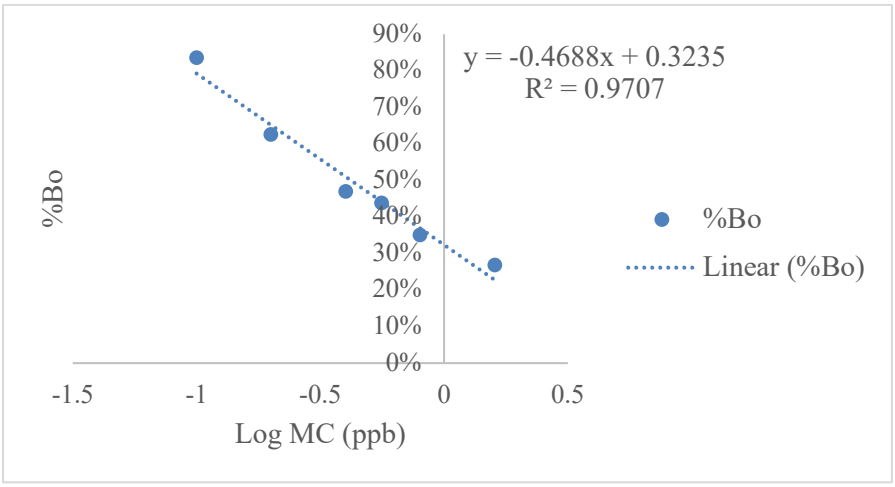
S5: Standard curves for qPCR for mcyA.



S6: qPCR melt curve



S7: Microcystin (ELISA) standard curve



S8: Correlations between mcyA gene expression and copies, chl-a and microcystin concentration data from Cayuga 2018 samples (without b.d. samples)

Cayuga 2018 samples (without b.d. samples)

	log chl-a(μg/L)	log DNA (copies/mL)	log RNA (copies/mL)
log DNA (copies/mL)	-0.384(n=19)		
log RNA (copies/mL)	0.399(n=13)	0.432(n=11)	
log MC (μg/L)	0.848**(n=16)	-0.51*(n=16)	0.585(n=11)

S9: Correlations between mcyA gene expression and copies, chl-a and microcystin concentration data from Cayuga 2018&2019 + Honeoye samples (with b.d. samples)

Cayuga 2018&2019 + Honeoye samples (with b.d. samples)

	log chl-a(μg/L)	log DNA (copies/mL)	log RNA (copies/mL)
log DNA (copies/mL)	-0.165(n=22)		
log RNA (copies/mL)	0.637**(n=16)	0.498*(n=18)	
log MC (μg/L)	0.869**(n=20)	-0.412(n=23)	0.793**(n=18)